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(19)

(54) PROCESS FOR PREPARING INJECTABLE COMPOSITIONS

(71) We, BEECHAM GROUP LIMITED, a British Company of Beecham House, Great West Road, Brentford, Middlesex, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a process for preparing finely divided micro-particles of tyrosine having a water-soluble dialdehyde-treated allergen dispersed therein for use in desensitisation therapy of persons who are liable to allergic reactions.

It has been proposed in desensitisation therapy to use a therapeutic composition comprising finely-divided solid micro-particles of a metabolisable substance having an active drug physically incorporated therein, that is suspended in a physiologically acceptable or non-toxic liquid carrier and capable of injection into the patient.

British Patent No. 1,377,074 (Application No. 32780/71), hereinafter called the parent patent, describes a process for preparing injectable compositions which consist of finely-divided micro-particles of tyrosine having an allergen dispersed therein, which process comprises mixing a solution of tyrosine in a strong aqueous acid with an aqueous or water-miscible solution of the desired allergen and simultaneously or subsequently neutralising the resultant solution whereby finely-divided micro-particles of tyrosine containing the allergen are precipitated; and subsequently separating the said micro-particles.

The parent patent refers quite generally to "allergens" and relates to a valuable method of preparing tyrosine micro-particles containing such materials. The process conditions are such that the process is effective only with water-soluble allergens and the parent patent describes several examples of such allergens.

British Patent No. 1,282,163 describes a method of preparing "modified allergens" which comprises the reaction of an allergenic extract with a polyaldehyde, a carbodiimide, an epihalohydrin or an inorganic cyanate, with the proviso that when an inorganic cyanate is employed the reaction is carried out under acid conditions. The allergenic extract which is modified in this process is obtained by extraction of an allergenic material with a suitable solvent, usually an aqueous solvent, followed by partial purification. The resultant modified allergens probably contain intra-molecular cross-linking with possibly some inter-molecular cross-linking, and have a reduced allergenicity relative to the unmodified allergen.

The modified allergens claimed in British Patent No. 1,282,163 are water-insoluble or only sparingly water-soluble. We have now found that water-soluble dialdehyde modified allergens may be prepared and used in the process of the parent patent to yield a product which advantageously combines efficacy with safety in desensitisation therapy. Such water soluble modified allergens represent a particular sub-class of water-soluble allergens which is not specifically described in the parent patent.

Accordingly, the present invention provides a process for preparing finely-divided micro-particles of tyrosine having a water soluble dialdehyde-treated allergen dispersed therein, which process comprises mixing (a) an aqueous solution of a water-soluble dialdehyde modified allergenic extract of an allergenic material with (b) a solution of tyrosine in a strong aqueous acid; and simultaneously or subsequently neutralising such mixture of solutions, thereby precipitating the required finely-divided micro-particles of tyrosine having a water soluble dialdehyde-treated allergen dispersed therein.

The solution of modified allergen used in this process may either be obtained directly by reacting an aqueous solution of an extract of an allergenic material with a dialdehyde under substantially neutral conditions or by making up a solution of a dried modified allergen previously prepared by such a process.

Preferably the dialdehyde is glutaraldehyde.

The allergenic extract for present use is an extract of an allergenic material such as, for example, an extract of one or more pollens, or of house dust, or of the house dust mite *Dermatophagoides pteronyssinus* which occurs in hour dust, or of moulds, or of animal hair, dander or fur. The extract may be prepared by any of the conventional ways for preparing allergen extracts and, if desired, the allergen material so extracted may be purified by precipitation, dialysis or gel filtration.

The allergenic extract is modified by treatment with a dialdehyde, preferably glutaraldehyde, at pH 7 ± 1 (and the resultant product may be isolated in solid form at this stage if so desired).

A solution of the water-soluble dialdehyde-treated allergenic extract at pH 7 ± 1, obtained either as the reaction mixture from the modification process or from the solvation of a solid modified allergenic extract, is then mixed with a solution of tyrosine in a strong aqueous acid. This step is carried out as described in the parent patent and the strong acid is usually an inorganic acid, preferably hydrochloric acid.

The resulting mixture of solutions of modified allergen and tyrosine is neutralised as described in the parent patent. By neutralisation is meant an adjustment of pH to a value within the range 4.0 to 7.5. This neutralisation can be carried out subsequently to the mixing of the two aforesaid solutions. However, it is important that, at no time, or at least at no prolonged time, during the neutralisation does the pH of the solution rise appreciably above 7.5. This condition can be met by vigorous stirring of the solution and by the use only of the required amount of base, if desired. Various buffering agents can usefully be added to the solutions of modified allergen to assist in pH control during the mixing and neutralisation stages.

A particularly useful method of carrying out the neutralisation is for separate streams of the solution of tyrosine in acid and the neutralising base to be run into the solution of modified allergen. The rates of flow of the added solutions being controlled by pH-stat, that is by equipment which regulates the flow of one or both of the solutions so that the pH of the reaction mixture remains substantially constant at a predetermined level. We have found that optimum results are usually obtained by pH control within the range 6.5 to 7.5 though the precise pH may vary according to the nature of the allergen.

The result of the neutralisation is the immediate precipitation of the tyrosine as microfine particles within and/or upon which the solution of modified allergen is occluded and/or adsorbed. After the precipitation the mixture is either washed immediately or allowed to stand for a period of from a few hours to a day or two prior to washing. Desirably the precipitate is obtained as fine as possible and this is achieved by rapid neutralisation of the solution coupled with vigorous agitation while this is being carried out.

As described in the parent patent the resulting precipitate of micro particles of tyrosine containing the modified allergen may be removed from the solution by centrifugation or filtration and washed, e.g. with phenol-saline, before being resuspended in a physiologically-acceptable carrier such as phenol-saline, or sterile water, to produce an injectable composition suitable for use in desensitisation therapy.

The materials produced by the process of this invention are believed to be novel, and as such form part of the invention. Accordingly in a further aspect the invention also provides finely-divided micro-particles of tyrosine having a water soluble dialdehyde-treated allergen disposal therein. From the preceding paragraph it will be appreciated that the invention also provides an injectable pharmaceutical composition suitable for use in desensitisation therapy, which composition comprises finely-divided micro-particles as hereinbefore described and a physiologically acceptable carrer.

The following Examples illustrate the present invention:

Example 1

Tyrosine having modified grass pollen dispersed therein

14 mls. of a neutral solution of approximately 2 mgm/ml dry weight of grass pollen extract which had been partially purified by dialysis or fractionation was chemically modified by the addition of an equal volume of 0.25% w/v purified glutaraldehyde and the mixture stirred for a period of approximately 2 hours. To the above mixture was added 10 mls of a phosphate buffer solution pH 7 ± 1, following by the simultaneous addition of 10 mls of L-tyrosine (prepared by dissolving 24 gm. L-tyrosine to 100 mls in 3.8 N hydrochloric acid) and 10 mls of 3.2 N sodium hydroxide, with vigorous agitation at pH 7 ± 1. The suspension so formed was centrifuged, washed repeatedly with buffered saline to remove contaminants and resuspended in buffered phenol saline pH 6 ± 1 to a volume of 60 mls.

Example 2

Tyrosine having modified Bermuda grass pollen dispersed therein.

14 mls of a neutral solution of 10% w/v Bermuda grass pollen extract which had

been partially purified by dialysis or fractionation was chemically modified by the addition of an equal volume of 1% w/v purified glutaraldehyde and the mixture stirred for a period of approximately 2 hours. Unreacted glutaraldehyde was removed by dialysis.

To the fluid retentate was added 10 mls of a buffer solution of pH 7±1, followed by the simultaneous addition of 10 mls of L-tyrosine (prepared by dissolving 24 gms of L-tyrosine to 100 mls in 3.8 N hydrochloric acid) and 10 mls of 3.2 N sodium hydroxide with vigorous agitation at pH 7±1. The suspension so formed was centrifuged, washed repeatedly with buffered saline to remove contaminants and resuspended in buffered saline pH 6±1 to a volume of 60 mls.

Example 3

Tyrosine having modified cultivated rye pollen dispersed therein.

The process as described in Example 2 was repeated but as allergen there was used a 10% w/v cultivated rye pollen extract.

Example 4

Tyrosine having modified tree pollen dispersed therein.

The process as described in Example 2 was repeated but as allergen there was used a 10% w/v tree pollen extract.

Example 5

Tyrosine having modified grass pollen dispersed therein.

A process as described in Example 2, but as allergen there was used a 10% grass pollen extract.

Example 6

Tyrosine having modified short ragweed pollen dispersed therein.

A process as described in Example 2 but as allergen there was used a 6% short ragweed pollen extract and the purified glutaraldehyde was 0.6% w/v.

Example 7

Tyrosine having modified *D. pteronyssinus* dispersed therein.

12 mls. of a neutral solution of 10% *D. pteronyssinus* extract which had been partially purified by dialysis or fractionation was chemically modified by the addition of an equal volume of 4% w/v purified glutaraldehyde and the mixture stirred for a period of approximately 2 hours. Unreacted glutaraldehyde was removed by dialysis.

To the fluid retenate was added 10 mls. of a buffer solution of pH 7±1, followed by the simultaneous addition of 10 mls. of L-tyrosine (prepared by dissolving 24 gm. of L-tyrosine to 100 mls. in 3.8 N hydrochloric acid) and 10 mls. of 3.2 N sodium hydroxide, with vigorous agitation at 7±1.

The suspension so formed was centrifuged washed repeatedly with buffered saline to remove contaminants and resuspended in buffered saline pH 6±1 to a volume of 60 mls.

The products of Examples 1 to 7 are pharmaceutically acceptable and are suitable for use in desensitisation therapy as injectable compositions.

WHAT WE CLAIM IS:—

1. A process for preparing finely-divided micro-particles of tyrosine having a water soluble dialdeyhde-treated allergen dispersed therein, which process comprises mixing (a) an aqueous solution of a water-soluble dialdehyde modified allergenic extract of an allergenic material with (b) a solution of tyrosine in a strong aqueous acid, and simultaneously or subsequently neutralising such mixture of solutions, thereby precipitating the required finely-divided micro-particles of tyrosine having a water soluble dialdehyde-treated allergen dispersed therein.

2. A process according to claim 1 wherein the aqueous solution of modified allergen is obtained directly by reacting an aqueous solution of an extract of allergenic material with a dialdehyde under neutral conditions.

3. A process according to either claim 1 or 2 wherein the dialdehyde is glutaraldehyde.

4. A process according to any of the preceding claims wherein the strong aqueous acid employed in stage (b) is hydrochloric acid.

5. A process according to any of the preceding claims, wherein sodium hydroxide is employed to neutralise the reaction mixture.

6. A process according to any of the preceding claims wherein a buffer is added to the reaction mixture before the neutralisation procedure is carried out.

7. A process according to any of the preceding claims, wherein the neutralising base and the solution of tyrosine in acid are run into the solution of modified allergen simultaneously.

8. A process according to claim 7, which is carried out at substantially constant pH by regulation of the rates of simultaneous addition of the solution of tyrosine and neutralising solution to the solution of modified allergen.

9. A process according to any of the preceding claims, wherein the allergen is an extract of one or more pollens or of house dust or of house dust mites.

10. A process for preparing finely-divided micro-particles of tyrosine having a water soluble dialdehyde-treated allergen dispersed therein substantially as hereinbefore described with reference to Example 1.

11. A process for preparing finely-divided micro-particles of tyrosine having a water soluble dialdehyde-treated allergen dispersed therein substantially as hereinbefore described with reference to any one of the Examples 2 to 7.

12. Finely-divided micro-particles of tyrosine having a water soluble dialdehyde-treated allergen dispersed therein.

13. Micro-particles as claimed in claim 12, wherein the dialdehyde is glutaraldehyde.

14. Micro-particles as claimed in claim 12 or 13, wherein the allergen is an extract of one or more pollens.

15. Micro-particles as claimed in claim 14, wherein the allergen is an extract of one or more grass pollens.

16. Micro-particles as claimed in claim 14, wherein the allergen is an extract of ragweed pollen.

17. Micro-particles as claimed in claim 12 or 13, wherein the allergen is an extract of house dust or of house dust mites.

18. Micro-particles as claimed in claim 12, substantially as hereinbefore described with reference to Example 1.

19. Micro-particles as claimed in claim 12, substantially as hereinbefore described with reference to any one of the Examples 2 to 7.

20. Micro-particles as claimed in claim 12, whenever prepared by a process as claimed in any one of the claims 1 to 11.

21. An injectable pharmaceutical composition suitable for use in desensitisation therapy which composition comprises micro-particles as claimed in any one of the claims 12 to 18, and a physiologically acceptable carrier.

22. A composition as claimed in claim 21, substantially as hereinbefore described with reference to Example 1.

23. A composition as claimed in claim 21, substantially as hereinbefore described with reference to any one of the Examples 2 to 7.

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